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Alterations in Mitochondrial State 4→3 Transition Underlie Stress-Induced Energetic-Redox Imbalance and Myocyte Dysfunction in Diabetic Mice
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Diabetes is a syndrome in which mitochondrial alterations may participate in the dysfunction, and ultimately failure of several organs. The diabetic heart in particular can be subjected to both glycemic level oscillations and increased workload (via β-stimulation), events that may further deteriorate its mechanical properties. Here we show that when diabetic heart (*db/db*) cells are challenged with high levels of extracellular glucose (30 mM) and concomitant β-adrenergic stimulation via isoproterenol (ISO), intracellular GSH is greatly diminished while superoxide and H_2O_2 generation substantially increased. The typical enhancement in sarcomere shortening and whole Ca^{2+} transient driven by β-adrenergic stimulation is blunted in *db/db* cells, along with hampered relaxation and Ca^{2} reuptake. Mitochondria isolated from *db/db* hearts subjected to high-glucose/ISO regimen exhibit markedly decreased coupling ratios from respiratory complexes I, II, and IV while ROS emission is greatly increased, under both forward and reverse electron transport. These changes reflect a profound alteration in the crucial (energy supplying) state 4→3 transition that ultimately results in lowered ATP synthesis and in a "non-stop" reactive oxygen species (ROS) emission in the presence of ADP. Incubating high-glucose/ISO treated *db/db* myocytes with cell-permeable GSH restores intracellular GSH, blunts ROS production and fully rescues contractility/relaxation in these myocytes. These results show the direct role played by an oxidized redox environment in triggering the negative synergy among mitochondrial ROS and energetics leading to the E-C coupling impairment. Our study maps mitochondrial sites, i.e. state 4→3 transition, that in diabetic heart cells account, at least in part, for excess ROS emission and loss in energy supply when metabolically and energetically challenged.

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The Role of Reactive Oxygen Species in Contractile Dysfunction Following Ischemia/Reperfusion
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Myocardial stunning occurs following ischemia-reperfusion, and is characterized by depressed contractility and elevated resting tension that is distinct from dysfunction associated with tissue damage or death. A wide variety of factors have been shown to contribute to post-ischemic myocardial dysfunction including cytosolic Ca^{2+} overload, acidosis, and the damaging effects of degradative enzymes and free radicals such as reactive oxygen species (ROS). These mechanisms act over a wide range of timescales. Intracellular Ca^{2+}, Na^{+}, and pH return to near pre-ischemic levels within a matter of minutes, while ROS production persists for up to three hours. The symptoms of myocardial stunning can persist even longer, indicative of a modified redox environment in the myocyte. Studies have revealed a number of sources of ROS production during reperfusion, but the mitochondria's role in regulating the balance of electron-carrying redox couples implicates cellular respiration as a critical source of ROS. In the modeling work presented here, we link a mathematical model of mitochondrial respiration and ROS production with a model of excitation-contraction coupling in the guinea pig myocyte. Experimentally determined ROS-dependent modulation of ryanodine receptors, sarcoplasmic reticulum (SR) Ca^{2+} ATPase, Na^{+}-K^{+} ATPase and myofilaments is incorporated into our model. Using this integrated model, we show that contractile dysfunction associated with myocardial stunning persists after ROS-mediated alterations in protein function. ROS-related changes in Na^{+}-K^{+} ATPase flux lead to altered intracellular Na^{+}, which modulates diastolic intracellular Ca^{+} via the Na^{+}-Ca^{2+} exchanger. Reduced SR Ca^{2+} ATPase activity and increased ryanodine receptor leak cause decreased SR Ca^{2+} load. Finally, ROS-related alteration of myofilament proteins reduces their Ca^{2+} sensitivity, which also contributes to decreased contractile force. Taken together, all of these mechanisms lead to contractile dysfunction caused by mitochondria-derived ROS.

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Ec Coupling is Preserved in Ncx Knockout Mice Exposed to Metabolic Inhibitors
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To determine whether ablating the cardiac sodium-calcium exchanger (NCX) prevents couplon loss during metabolic inhibition (MI), we recorded Ca transients or resting Ca spark frequency (CaSpF) in ventricular myocytes isolated from WT and ventricular-specific NCX knockout (KO) mice. In field stimulated myocytes loaded with 10 μM Fluo-3 AM, application of the mitochondrial and glycolytic inhibitors FCCP (50 nM) and 2-DG (10 mM) reduced Ca transients by 54.2 ± 3.3% in WT but only by 19.0 ± 6.0% in KO ($p < 0.05$). Similarly, MI suppressed action potential evoked Ca sparks in patch clamped WT myocytes loaded with 1 mM fluo-3 salt by 60.0 ± 8.2% ($p < 0.05$), but in KO by only 33.0 ± 3.4%. The reduction in ICa during MI was similar for both WT and KO and SR Ca stores remained at control levels in both cell types during MI. In patch clamped myocytes loaded with 1 mM K5Fluo-3 and held at −75 mV, MI reduced resting CaSpF in WT by 92.4 ± 6.7%, ($p=0.008$) but had no effect on CaSpF in KO Abolishing Na and Ca gradients by using the same "internal" solution for both the bath and pipette (current clamp mode, VRest = 0 mV), eliminated the KO's resistance to MI, causing CaSpF to decline to a similar extent in both WT and KO (59.0 ± 6.2% and 60.7 ± 10.9%, respectively). This finding suggests that elevated diadic cleft Ca, a major characteristic of KO myocytes (Pott et al, Biophys J, 92: 1431, 2007; Neco et al. Biophys J, 99: 755, 2010), maintains effective triggering of SR Ca release during MI despite reduced ICa.

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Mechanisms Regulating the Organellar Ca^{2+} Dynamics in Intact Hearts During Ischemia-Reperfusion
Dmytro Kornyeyev, Azade D. Petrosky, Ariel L. Escobar.
The accumulation of Ca^{2+} during ischemia, in both cytosol and sarcoplasmic reticulum (SR) of ventricular cardiomyocytes, contributes to the condition known as myocardial stunning. This not only disrupts normal Ca^{2+} dynamics in the contractile cells but also makes them more prone to apoptosis and/or necrosis. Therefore, the identification of the mechanisms underlying this phenomenon is important for understanding functional changes that occur during cardiac infarction. We examined the role of acidosis, inhibition of Na^{+}/Ca^{2+} exchanger (NCX) and altered restitution of Ca^{2+} release as factors that can potentially be involved in the intracellular Ca^{2+} accumulation. Pulsed Local Field Fluorescence experiments on Langendorff-perfused mouse hearts reveal that a decrease in Tyrode solution's pH of from 7.4 to 6.2 diminished the amplitude of Ca^{2+} transients (52 ± 3% of control) without elevation of diastolic Ca^{2+} level (measured with rhod-2 for cytosol and mag-fluo-4 for intra-SR). This suggests that acidosis alone cannot be responsible for accumulation of Ca^{2+} in myocytes. The combination of low pH (6.2) and partial (25%) replacement of Na^{+} with Li^{+} (to inhibit NCX) resulted in 1.4-fold increase in resting diastolic [Ca^{2+}] in cytosol suggesting that the NCX activity might contribute to ischemia-induced changes in intracellular Ca^{2+} content. The restitution of Ca^{2+} release, measured with double-pulse protocols, slowed down during the ischemic period. Under these conditions, the decrease of Ca^{2+} release from SR might promote an accumulation of Ca^{2+} in this cellular compartment. Simultaneous measurements of intracellular action potentials (AP) and Ca^{2+} transients revealed that the restitution of AP is not the limiting step for Ca^{2+} release restitution during ischemia. Interestingly, the slow restitution persisted during reperfusion leading to an amplification of Ca^{2+} alternans. The study was supported by NIH R01-HL-084487 grant.

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Optical Measurement of Action Potential in Adult Ventricular Myocytes
Qinghai Tian, Martin Oberhofer, Sandra Ruppenthal, Anke Scholz, Volker Buschmann, André Zeug, Peter Lipp, Lars Kaestner.
Due to their shape and fragility primary cells such as neurons or cardiomyocytes cannot enter automated membrane potential measurements on patch-clamp robots. Instead contact-free optical approaches would be advantageous. So far procedures based on potentiometric dyes are lacking one or several of the following properties: (i) ability for quantification, (ii) sufficiently fast response time to follow action potentials (APs), (iii) sufficiently high signal-to-noise ratio to avoid signal averaging, (iv) sensor-induced alterations of AP properties. We show that the small molecule dye di-8-ANEPPS and the novel genetically encoded sensor "Mermaid" provide quantitative potential information. Based on an improved optical design, individual AP could be recorded with an exceptional signal-to-noise-ratio. The sensors were validated using the patch-clamp technique, confocal microscopy and fluorescence lifetime imaging in combination with global unmixing procedures. When applying such approaches we identified distinctly different pharmacological profiles of APs between adult and neonatal cardiomyocytes. We concluded that optical recordings of individual APs can be performed using small molecule dyes and novel genetically encoded biosensors without disturbing the properties of APs. Additionally we have demonstrated the superiority of adult over neonatal cardiomyocytes for pharmacological investigations such as QT-screens.

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The Differential Action of Cytocalasin D in T-tubular Remodelling of Ventricular Myocytes
Lars Kaestner, Qinghai Tian, Sara Pahlavan, Katharina Oleinikow, Sandra Ruppenthal, Anke Scholz, Martin Oberhofer, Christian Schumann, Annette Kraegeloh, Peter Lipp.
Cytocalasin D (cytoD) is a fungal metabolite that inhibits cytokinesis by blocking formation of contractile microfilament structures resulting in multinucleated cells, reversible inhibition of cell movement, and the induction of